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Development and characterization of co-loaded curcumin/triazole-halloysite systems and evaluation of their potential anticancer activity

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ABSTRACT

Positively charged halloysite nanotubes functionalized with triazolium salts (f-HNT) were employed as a carrier for curcumin molecules delivery. The synthesis of these f-HNT new materials is described. Their interaction with curcumin was evaluated by means dynamic light scattering (DLS) and UV–vis spectroscopy in comparison with pristine unmodified HNT (p-HNT). The curcumin load into HNT was estimated by thermogravimetric analysis (TGA) measurements, while the morphology was investigated by scanning electron microscopy (SEM) techniques. Release of curcumin from f-HNT, at three different pH values, by means of UV–vis spectroscopy was also studied. Furthermore, different cancer cell lines were used to evaluate the potential cytotoxic effect of HNT at different concentrations and culture times. The results indicated that the f-HNT drug carrier system improves the solubility of curcumin in water, and that the drug-loaded f-HNT exerted cytotoxic effects against different cell lines.

1. Introduction

Phenolic compounds originated from one of the main class of secondary metabolites in plants, are natural phytochemicals mostly deriving from phenylalanine, which are widely present in food and nutraceuticals (Aggarwal et al., 2008). Among them, curcumin and its derivatives have been extensively studied and evaluated for their biological activity.

Curcumin [bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione] is a natural phenolic compound isolated as a yellow pigment from the dried rhizome of *Curcuma longa*, a plant that is widely cultivated in tropical areas of Asia and Central America, commonly used as a spice, as a food colorant, and even as a food preservative. Several studies, also, report that it is an agent possessing a wide variety of biological and pharmacological activities, including anti-proliferation (Choi et al., 2006), anti-apoptosis (Aggarwal et al., 2008), anti-angiogenesis (Lin et al., 2007) and inhibition of cell invasion and metastasis (Chen et al., 2008).

Successful application of this compound is hampered, however, by the occurrence of some disadvantageous properties. Being hydrophobic in nature, curcumin is sparingly soluble in water (ca. 0.6 μg/mL). Moreover, it degrades rapidly under neutral or alkaline conditions, with a half-life shorter than 10 min in phosphate buffer solution at pH 7.2 (Kurien et al., 2007). As a result, its bioavailability is poor, particularly after oral or topical administration (Anand et al., 2007). Therefore, a carefully designed carrier could significantly facilitate curcumin delivery and broaden the range of its possible pharmaceutical applications.

Nanoscale drug delivery systems are an innovative approach for overcoming the aforementioned problems. Previous attempts at encapsulating curcumin in liposomes, phospholipid complexes, or other nanoparticle-based technologies have been reported, showing an improvement in water dispersibility and a longer circulation time (Tang et al., 2010; Duan et al., 2010; Kim et al., 2011)

A possible nanosized delivery system is constituted by naturally available clay halloysite nanotubes (HNT). Halloysite has been found a viable and inexpensive nanoscale container for the encapsulation of biologically active molecules such as biocides and drugs, as Price et al. (2001) first demonstrated.

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Halloysite is a hydrated polymorph of kaolinite consisting of silica on the outer surface and alumina at the innermost surface of the tubular structure (Bates et al., 1950). Therefore, the different inner and outer-compositions of these materials allow to accomplish different chemical reactions on either surface (Massaro et al., 2014a,b; Li et al., 2008; Du et al., 2010; Wang et al., 2008; Vergaro et al., 2010; Lvov et al., 2002; Matsuno et al., 2003, 2006; Ma et al., 2011, 2012; Terayama et al., 2010; Horiuchi et al., 2009).

The functionalization of HNT with organic salts is a good strategy to prepare a new class of materials presenting most of the features and properties of organic salt, allowing them to be dispersed in physiological media.

For the cellular internalization of nanoparticles in general, surface charge is, also, important in determining whether the nanoparticles would cluster in blood flow or would adhere to, or interact with oppositely charged cells membrane (Feng, 2004). A cationic surface is desirable as it promotes the interaction between the nanoparticles and the cells, and hence increases the rate and extent of internalization (Shenoy and Amiji, 2005).

In the last years triazolium derivatives have attracted the attention of scientists because the *N*-alkyl-1,2,3-triazoles moiety is easily accessible through the versatile copper-catalyzed (CuAAC) [2 + 3] cycloaddition (Huisgen, 1963; Meldal and Tornoe 2008; Finn and Fokin, 2010).

Triazoles and their derivatives, indeed, occupy a central position (Noël et al., 2009) amongst the most significant compounds that constitute pharmaceutically and medicinally important drug centers (Al-Masoudi et al., 2006). Several triazoles have been reported to possess antibacterial (Singh et al., 2006), antifungal (Rezaei et al., 2009), antitumor (Guo-Qiang et al., 2008), plant growth regulating (Jin et al., 2007) and cytotoxic (Bagihalli et al., 2008) activities.

In this work we report the selective functionalization of halloysite nanotubes at the external surface with triazolium salts and the interaction of these nanomaterials with curcumin. The functionalization served for two purposes: (i) introducing a positive charge on the halloysite carrier; (ii) verifying whether the presence of the biological active triazolium moiety could exert a synergic effect with curcumin.

2. Experimental

2.1. Materials and methods

All reagents and materials needed were used as purchased (Aldrich), without further purification.

Halloysite was supplied by Applied Minerals. This material has an average tube diameter of 50 nm and inner lumen diameter of 15 nm. Typical specific surface area of this halloysite is 65 m^2/g; pore volume of ~1.25 mL/g; refractive index 1.54; and specific gravity 2.53 g/cm^3.

The 3-azidopropyltrimethoxysilane was synthetized as previously reported (Karl and Buder, 1983).

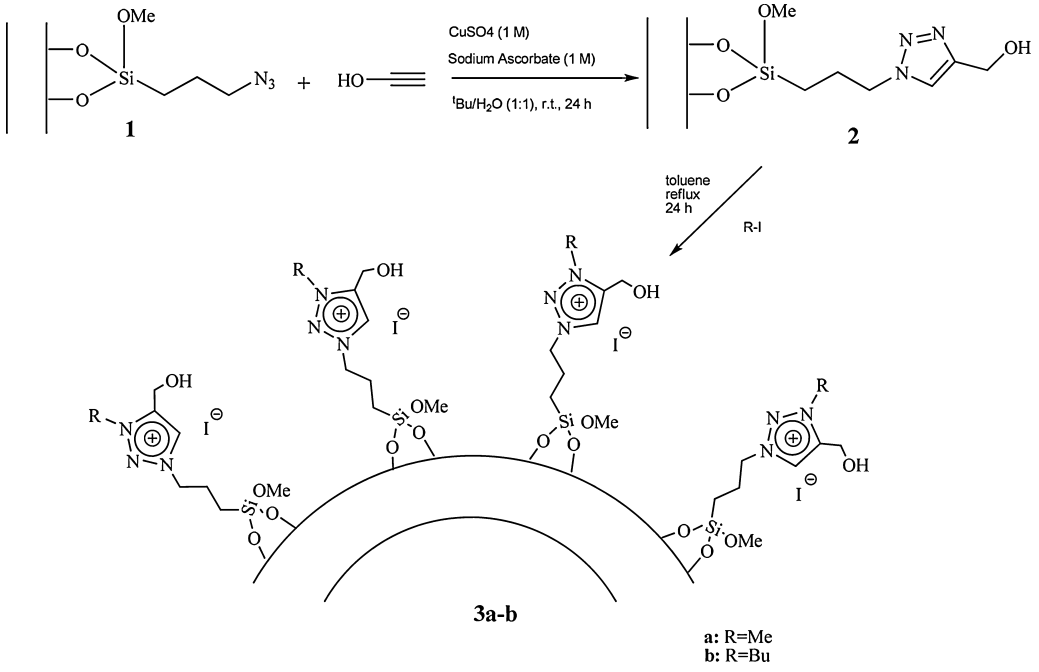
UV–vis spectra were recorded on a Beckmann DU 650 spectrometer.

IR spectra were recorded with an Agilent Technologies Cary 630 FTIR spectrometer. Specimens for measurements were prepared by mixing 5 mg of the sample powder with 100 mg of KBr.

An ESEM FEI QUANTA 200 F microscope was used to study the morphology of the functionalized HNTs. Before each experiment, the sample was coated with gold under argon by means of an Edwards Sputter Coater S150A to avoid charging under the electron beam.

Thermogravimetric analyses were performed on a Q5000 IR apparatus (TA Instruments) under a nitrogen flow (25 $cm^3\,min^{-1}$ for the sample and 10 $cm^3\,min^{-1}$ for the balance). The weight of each sample was ca. 10 mg. The measurements were carried out by heating the sample from room temperature up to 900 °C at a rate of 10 °C min^{-1}. The difference between the thermoanalytical curve of loaded HNT or f-HNT and the corresponding pristine nanoparticles was calculated, in order to highlight the degradation of the curcumin present in the composites.

The DLS measurements were performed at 22.0 ± 0.1 °C in a sealed cylindrical scattering cell at a scattering angle of 90°, by means of a Brookhaven Instrument apparatus composed of an BI-9000ATcorrelator and a He–Ne laser (75 mW) at a wavelength (λ) of 632.8 nm. The solvent was filtered by means of a Millipore filter with 0.45 μm pore size. For all systems, the field-time



Scheme 1. Synthesis of functionalized f-HNT**3a-b**.